Sex and age-biased nematode prevalence in reptiles

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Abstract

Prevalence and intensity of parasitic infections is often higher in male than in female vertebrates. This bias may represent either differences between host sex in exposure or susceptibility to parasites. The former may be due to sex-specific behaviour of the host, including differential habitat use or diet. Differences in susceptibility are often regarded as a negative effect of male sex-steroid hormones on the immune system. Host-parasite dynamics are of great interest in terms of reptile survival, ecology and conservation. We used, for the first time, molecular diagnostics to track nematode parasitism in wild populations of reptiles non-invasively. Using slow worms (*Anguis fragilis*) as a model species, we investigated the interacting effects of time of year, sex, length, weight and climatic variables on the prevalence of the gastroenterological parasitic nematode *Neoxysomatium brevicaudatum*. Faeces were collected from three sites over two years. There was an interaction between sex and time of year, with lower nematode prevalence in males than females in July or August (different between years) but a high prevalence in males in April. As the latter is during the slow worm breeding season, this may be the result of testosterone-induced immunosuppression. A second order interaction between slow worm length and weight was found to be significant, with a positive association between prevalence and body condition in young slow worms and a negative association in older slow worms. The convex pattern of nematode prevalence with age that emerged suggests an increase with age-related exposure and a decrease with age-related acquired immunity.

Introduction

Globally, parasites represent a major source of disease and mortality in reptiles (reviewed in Gibbons *et al.* 2000). The dynamics of such host-parasite interactions are therefore of great interest in terms of reptile survival, ecology, conservation and species management. In vertebrates, the prevalence (percent of hosts infected) and intensity (number of parasites per host) of parasitic infections are frequently found to be higher in males than in females (Poulin 1996a; Zuk & McKean 1996; Schalk & Forbes 1997; Klein 2004; Robinson *et al.* 2008). This bias is usually explained as a function of either differential susceptibility (Zuk 1990; Poulin 1996a; Klein 2004; Moore & Wilson 2002) or exposure (Drobney *et al.* 1983; Tinsley 1989; Reimchen & Nosil 2001; Krasnov *et al.* 2005) between host sexes.

Differences in susceptibility are often attributed to the well documented negative association between sex-steroid hormones (particularly testosterone) and the immune system (Folstad *et al.* 1989; Folstad & Karter 1992; Wedekind & Jacobson 1998). The production of male secondary sexual traits is governed by testosterone, which is simultaneously immunity suppressive. Due to this testosterone-induced immunosuppression (Zuk & McKean 1996) the ability to display secondary sexual traits is costly (in terms of increased parasite risk) and therefore an honest signal of male quality, as only the fittest males are able to display with the burden of this handicap (Zahavi 1975; Kurtz & Sauer 1999). Host androgens not only have indirect effects on parasite abundance via the immune system, but they can also directly affect growth and development of the parasite itself (Drutz *et al.* 1981; Harder *et al.* 1992; reviewed in Lawrence 1991 and Beckage 1993).

Differences in exposure to parasites may be due to sex-specific behaviour of the host, including differential use of habitat between sexes (Tinsley 1989),

aggression between males for mating opportunities (Mills *et al.* 1999), aggregation of one sex (Zuk & McKean 1996), or differences in diet (Thomas 1965; Kennedy 1968; Borgstrom 1970; Drobney *et al.* 1983; Poole *et al.* 1983). Furthermore, behavioural differences in one sex which result in stress (e.g., territory defense, intraspecific fighting, energy-intensive courtship displays) may have significant negative effects on the immune system (Stein & Schleifer 1985), increasing susceptibility, if not exposure, to parasitic infection. Environmental and social factors which cause stress (e.g. temperature, light intensity, annoyance, crowding and hunger) have all been shown to cause higher parasite loads (Noble 1961, 1962, 1966; reviewed in Wolinska & King 2009).

Alternatively, exposure is not limited to behaviour and can be a result of sexual dimorphism. As males are often larger than females they may simply offer a larger contact area for parasites (Kuris *et al.* 1980; Hamann *et al.* 2006) or may ingest greater amounts of infected prey (intermediate hosts) (Poulin 1996b).

The age of the host can also influence the likelihood of parasitism. If parasites accumulate with time then a linear pattern of parasite load with age is to be expected (Hudson, Newborn & Dobson 1992). However, a convex age-infection curve is not uncommon (Kisielewska & Zubczewska 1973; Liat & Pike 1982; Gregory, Montgomery & Montgomery 1992). Younger hosts will have had less exposure time, but this increases with age, leading to maximum levels of parasitism in hosts of an intermediate age. Parasitism then decreases in older animals and this has been attributed to either a reduction in exposure to parasites through age-related changes in behavior (Tinsley 1989) or increased susceptibility through age-related changes in immunity (Streter *et al.* 1995).

There are plenty of confounding factors to consider, however. For example, if a host accumulates parasites with time and one sex has a higher mortality than the other (Paling 1965; Halvorsen & Andersen 1984) then the high parasite levels of the longer-surviving sex may be misinterpreted as a sex-bias. Season or year, too, may affect susceptibility to parasites, with differences in behaviour influencing exposure (Schall & Marghoob 1995*),* in particular changes in activity or diet during the breeding season (Drobney *et al.* 1983). Age and season, then, may need to be accounted for in order to draw meaningful conclusions, but this has seldom been the case.

While nematodes are the most abundant animals on earth (Coghlan 2005), they are generally considered to be one of the most difficult to identify. Due to a lack of informative features, many cannot be identified even to family level by skilled nematode taxonomists (McKeand 1998). Even where nematodes can be identified, quantifying parasitic infection under the microscope is a slow, laborious, tedious procedure. These problems can be resolved by using parasite species-specific PCR primers (Verweij *et al.* 2001; Zarlenga *et al.* 2001; Harmon *et al.* 2007). Such an approach has been used for detecting gastroenterological parasites in fish (Fang *et al.* 2010), birds (Zhao *et al.* 2009) and mammals (Al-Sabi *et al.* 2010; Marra *et al.* 2010) but has most commonly been devised for veterinary diagnosis in livestock (Zarlenga *et al.* 1998, 2001; Hung *et al.* 1999; Schnieder *et al.* 1999). Reptile parasites have not been studied in the wild using faecal analysis by PCR before nor, to our knowledge, have nematodes been detected or tracked in this way in any animals, captive or wild. The use of PCR primers, once optimised, offers a fast, repeatable and reasonably cost-effective means for standardised screening of faecal samples without some of the constraints of traditional visual screening and could compliment or replace

conventional means of examining a broad range of ecological topics, including, parasite transmission, parasite-host coevolution, immunity, impact on host behaviour or body condition, and parasitism affected by population fragmentation, host-reintroductions or climate change. The approach is especially useful for the ongoing monitoring of populations and for large scale studies which necessitate greater sample sizes and benefit from standardised procedure.

Here we describe the first application of species-specific PCR primers for investigating gastroenterological nematode parasitism (by *Neoxysomatium brevicaudatum*) in reptiles, applied to studying wild populations of slow worms in the UK, a protected species. We examined whether there is a sex-bias in the prevalence of *N. brevicaudatum*, by molecular analysis of faecal samples, and to further examine the interacting effects of sex with time of year, length, weight and climatic variables on nematode prevalence. In doing so we tested the hypotheses that sex, growth stages and breeding cycles would all affect susceptibility to nematode infection. In particular, that: 1. Male slow worms are more prone to infection during the breeding season (as a possible result of immunosuppression) and 2. Parasitism increases with host age in males and females due to greater exposure to, and accumulation of, parasites.

Materials and Methods

Ecology of host and parasite

Neoxysomatium brevicaudatum is an intestinal parasite of amphibians and reptiles (Shimalov & Shimalov 2000; Shimalov *et al.* 2000; Kirin 2002; Karadeniz *et al.* 2005; Saglam & Arikan 2006). It commonly parasitises herpetofauna throughout

Europe and has been identified in UK slow worm populations with a mean intensity of 70.9 nematodes per host, ranging from 1 to 686 (Jones *et al.* 2012). The nematode has a direct life-cycle: eggs hatch outside of the host, first stage larvae moult twice to the infective third stage which then infects the host either orally or by skin penetration (Vashetko & Siddikov 1999; Saeed *et al.* 2007). Dissections of Eastern European slow worms (the legless lizard *Anguis fragilis*) have revealed eleven species of parasitic helminths (Shimalov *et al.* 2000; Borkovcova & Kopriva 2005), with prevalence of *N. brevicaudatum* recorded as 11% (Shimalov *et al.* 2000) and 43% (Borkovcova & Kopriva 2005), but these findings are constrained by the small sample sizes involved: nineteen and seven respectively.

Slow worms are ovi-viviparous, with young born in an egg membrane that breaks after birth (Dely 1981). They are active between March-October with breeding taking place in April/May and young born in August/September (Smith 1990; Platenberg 1999). Juveniles reach sexual maturity at around 4-5 years (Smith 1990; Platenberg 1999). In the UK, slow worms typically have a biennial breeding cycle (Patterson 1983; Smith 1990; Platenberg 1999). Males display aggressive behaviour towards other males during the breeding season and compete with each other for mating opportunities with females (Capula *et al.* 1992). Slow worms are sexually dimorphic, with head size being larger for males than females for a given snout to vent length (Sos & Herczeg 2009). This is likely to be the result of sexual selection and the role of head structure in male-male combat, as seen in other reptiles (Vitt 1983). While males develop proportionately larger heads, females develop larger bodies than males overall (Sos & Herczeg 2009). Their diet is known to include a variety of invertebrates including earthworms, slugs, snails, pill millipedes and Lepidoptera larvae (Jensen *et al.* 2009; Brown *et al.* 2012).

Study sites and faecal collection

A total of 270 faecal samples were collected from slow worms during monthly visits to three sites (Caerphilly, Ringwood and Creech, Fig. S1) with different habitat characteristics between April–September in both 2007 and 2008. The Caerphilly site is an approximately five hectare area of marshy grassland, comprising purple moor grass (*Molinia caerulea*), tufted hair grass (*Deschampsia cespitosa*), gorse (*Ulex* spp.), bramble (*Rubus fruticosus*) and ferns (*Dryopteris* spp.) surrounded by areas of species-poor acid grassland. The Ringwood site consists of just under a hectare of unimproved grassland adjacent to Ericaceous heathland and coniferous woodland. Creech is an area of Ericaceous heathland comprising common heather (*Calluna vulgaris*), bell heather (*Erica* cinerea) and gorse (*Ulex* spp.).

The head of each slow worm, which has a unique pattern, was photographed to ensure that no individual was sampled twice. Faecal samples were collected into 2 mm microcentrifuge tubes by gentle palpatation of the animals and preserved in 70% ethanol (for up to 48 hrs) until they could be stored in a -20°C freezer. Effort was taken to collect faeces from males and females at each sampling time, both adults and subadults and, where possible, juveniles. Sampling effort was spread equally across sites and months. Snout-vent length and total weight were measured, and the presence / absence of a complete tail recorded as an indication of a predatory attack.

Nematode DNA

DNA was extracted from ten whole nematode specimens found in slow worm faeces using the DNeasy® DNA Tissue Kit (Qiagen) in accordance with the manufacturer's instructions. PCR primers LCO1490 (5'- GGTCAACAAATCATAAAGATATTGG -3') and

HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer *et al.* 1994) were used to amplify a 658 bp region of the cytochrome oxidase I (COI) mitochondrial gene, using the following conditions: 1X buffer, 2 mM $MgCl_2$, 0.1 mM dNTPs (Invitrogen), 0.5 µM of each primer, 0.45 U *Taq* polymerase (Invitrogen) and 5ng/µL of DNA with an initial denaturation at 94^{o}C for 3 min, 45 cycles of 94 ^{o}C for 30 s, 46 ^{o}C for 1 min and 72 ^{o}C for 1 min, and a final extension at 72 ^{o}C for 5 min. PCR primers rift (5'-GCGGCTTAATTTGACTCAACACGG-3') and 1500R (5'-GCTATCCTGAGGGAAACTTCG-3') (Tkach *et al.* 2006) were also used to amplify a 549 bp region of 18S rDNA using the same PCR conditions as above. PCRs were run on a Peltier Thermal Cycler (Bio-Rad Laboratories, CA, USA), amplification success was determined by gel electrophoresis visualized with ethidium bromide, and PCR products were sequenced with an ABI 3100 automated capillary DNA analyzer (ABI Prism model 3100, Beaconsfield, UK). Construction of a neighbour-joining tree with COI and 18S (accession number JF713457) sequences using MEGA allowed molecular confirmation of the nematode species as *Neoxysomatium brevicaudatum*.

PCR primers design and testing

COI sequences homologous to that for *N. brevicaudatum* from a range of nematode taxa (see Table S1) were acquired from the Genbank database and aligned in BioEdit 7.0.4.1 (Hall 1999) for the design of *N. brevicaudatum*-specific primers. NetPrimer (Biosoft International) was used to test primer sequences for potential primer-dimer and hairpins which would reduce primer efficiency. Of the primers designed, the COI-J-1764 (5'-TCTTAGATTTTGACTTTTGCCTACAG-3') and COI-N-1938 (5'-AGAACTAACACCAGCACAATGTAATC-3') primer pair, which amplifies a 174 bp fragment, were species-specific showing no cross-reactivity when tested for

amplification against a range of 22 species (Table S2), including the slow worm itself, potential prey (annelids, gastropods and arthropods), and nematode species. Optimised PCR conditions were: 1X buffer, 2 mM $MgCl_2$, 0.1 mM dNTPs (Invitrogen), 0.5 µM of each primer, 0.45 U *Taq* polymerase (Invitrogen) and 5ng/µL of DNA with an initial denaturation at 94°C for 5 min, 40 cycles of 94 °C for 30 s, 66 °C for 45 s and 72 °C for 45 s, and a final extension at 72 °C for 10 min. PCR was also optimised using a Multiplex (Qiagen) kit under the following conditions: 1X Master Mix, 0.2µM each primer and 5ng/µL of DNA with an initial denaturation at 95°C for 15 min, 35 cycles of 94 °C for 30 s, 66 °C for 90 s and 72 °C for 90 s, and a final extension at 72 °C for 10 min.

Primer sensitivity was established with both normal *Taq* and Multiplex. Nematode DNA concentrations (determined by Nanodrop ND-1000 Spectrophotometer) were 3, 0.6, 0.3, 0.06, 0.03, 0.006 and 0.003 ng/µL. Amplification success was determined by gel electrophoresis visualized with ethidium bromide. Primers were sensitive up to 0.03 µg/µL DNA with normal *Taq* and between 0.003-0.006 ng/µL with Multiplex mix. The accuracy of primer screening has previously been established in a parallel study by comparison with conventional microscopy of slow worm faeces, giving a 100% detection rate with no false positives (Jones *et al.* 2011).

Faecal screening and statistical analysis

The 270 slow worm faecal samples were extracted using the QIAamp® DNA Stool Mini Kit (Qiagen) in accordance with the manufacturer's instructions. *Neoxysomatium brevicaudatum*-specific primers COI-J-1764 and COI-N-1938 were used with normal *Taq* to amplify DNA from any *N. brevicaudatum* nematodes co-

extracted in the faeces. Positive and negative (water) controls were used in each PCR batch. Samples testing negative were rescreened using the Qiagen Multiplex kit. This combined approach was considered more cost-effective than sole use of the more expensive, but more sensitive, Multiplex kit approach.

The effects of slow worm length, weight, age class, sex, and presence/absence of tail, along with site, month, year, along with temperature, rainfall and sunshine (monthly averages) on prevalence of *N. brevicaudatum* were explored within a Generalised Linear Model (GLM) (see Table S3). Weight, length, temperature, rainfall and sunshine were treated as covariates and all other predictors as factors. Weather information was obtained from the Met Office. Second order interactions included were sex:month, sex:site and length:weight. A binomial error distribution was used with a logit link function. Post-hoc contrast analyses were performed to test pair-wise comparisons of group means between significant factor levels. All analyses were conducted in the R statistical package version 2.9.2.

Results

Overall prevalence of *N. brevicaudatum* was 66.4% (95% CI: 57.3-75.5%) and 56.7% (95% CI: 48.7-64.7%) in male and female slow worms respectively, which was not significantly different ($\chi^2_1 = 2.58$, $P = 0.108$).

Although sex alone did not affect prevalence, the interaction between month and sex was found to have a significant effect ($\chi^2_5 = 15.9$, $P = 0.007$). Post hoc contrast analysis revealed significantly higher prevalence in males compared to females for April ($t_{218} = 1.98$, $P = 0.05$) (Fig. 1). Additionally, prevalence in males in April was significantly higher than it was in July ($t_{218} = 2.72$, $P = 0.007$) and August

(t_{218} =3.32, P = 0.001). Conversely, prevalence in females did not change from month to month.

With 2007 and 2008 analysed separately, the interaction between month and sex was still significant (χ^2_{11} = 29.7, P = 0.002), however, due perhaps to the reduced power of the analysis, there was no longer a significant difference in prevalence between males and females in April for either year. Instead, post-hoc contrast analysis revealed significantly lower predicted prevalence in males compared to females in mid-summer (July 2007 (t_{204} = 2.34, P = 0.03) and August 2008 (t_{224} = 2.24, P = 0.02); Fig. 2). This effect was likely masked when 2007 and 2008 were pooled by the decrease in male prevalence happening in different summer months in each of the years.

An interaction between length and weight also had a significant association with the prevalence of nematodes (χ^2_1 =9.7, P = 0.0007). Slow worm size affected the predicted prevalence such that in smaller animals (juveniles and sub-adults) there was a positive relationship between prevalence and weight which switched to a negative relationship in larger animals (adults, Fig. 4). By using slow worm length as a surrogate for age, and controlling for weight, there is a clear curvilinear pattern of predicted prevalence increasing with age up until adulthood and then decreasing (Fig. 5).

No other terms were found to be significant and were removed from the final model.

Discussion

The PCR primers COI-J-1764 and COI-N-1938 were demonstrated to be species-specific for slow worm parasite *N. brevicaudatum*. Although we cannot preclude

cross-reactivity with any species we didn't empirically test against, only two species of nematode were found in UK slow worms and of those, and others we tested, the primers amplified only the target.

Using these primers we identified a significant effect of host sex on parasitic prevalence, but this was highly dependent upon time of year. Prevalence in males was significantly higher than in females in April (Fig. 1). Difference in parasite prevalence between sexes is generally considered a result of either differential exposure, often arising from behavioural differences (Drobney *et al.* 1983), or in susceptibility, due to differences in either the host's resistance to parasites or their ability to remove them (Zuk 1990). Prevalence of infection is often higher in males than in females in vertebrates of many classes including fish (Reimchen & Nosil 2001), amphibians (Dare & Forbes 2008), birds (Robinson *et al.* 2008), mammals (Krasnov 2005) and reptiles (Amo *et al.* 2005). The parasites involved with male-biased infection include protozoan (Amo *et al.* 2005), nematodes (Dare & Forbes 2008), trematodes (Robinson *et al.* 2008) and mites (Christe *et al.* 2007). With the rise in prevalence in males coinciding with the onset of the mating season, when testosterone levels are at their highest in reptiles (Watt *et al.* 2003; Wack *et al.* 2008), it is likely that testosterone-induced immunosuppression, and the resulting increased susceptibility to parasites, is driving higher infection in males at this time. Further work would be needed to confirm this.

With the two years analysed separately, prevalence in males was found to be significantly lower than in females in mid-summer of each year (Fig. 2). This result appears to have been masked in the combined analysis for both years by the trend having happened in a different month each year. This difference in prevalence between the sexes could be attributed to a disparity in habitat use by males and

females, or increasing/decreasing encounter rates with parasites. Both sexes share territories throughout the year and are often found together, but this does not necessarily dictate similar foraging behaviour or use of their territory.

There was a significant association between predicted nematode prevalence and an interaction between slow worm length and weight. In slow worms over 130 mm (defined as adults), lower weight (i.e. poorer condition) correlated with an increase in predicted prevalence (Fig. 3), suggesting *N. brevicaudatum* has a negative effect on slow worm growth and health. This is to be expected and has been reported for a range of nematode-infected hosts (Calvete *et al.* 2004; Irvine *et al.* 2006). Alternatively, slow worms with poorer body condition may be more prone to parasites, either through exposure or susceptibility. Curiously, in slow worms below 130 mm (i.e. juveniles, sub-adults and young adults) the reverse trend was found: for any given length prevalence increased with an increase in weight, that is, prevalence was positively associated with condition. It may be that in these younger animals, those with the greatest foraging success are increasing not only their intake of food but also their exposure to parasites. Conversely, it may be that healthier slow worms are simply better able to withstand parasitic infection, while those already in poor condition die as a result of infection and were therefore undetected.

With length taken as a surrogate for age, and controlling for weight, the prevalence pattern found for *N. brevicaudatum* (Fig. 4) shows a positive correlation with age in young individuals followed by a negative correlation in older animals. The initial rise in prevalence is characteristic of the majority of age-intensity and age-prevalence curves, which show a rapid accumulation of parasites with increasing exposure after the age at which an animal is first susceptible to infection (Quinnell 1992; Krasnov *et al.* 2006). Typically such curves reach an asymptote where infection

is balanced by parasite mortality. The subsequent decrease in prevalence with age is also common (Sreter *et al.* 1995; Ladeia-Andrade *et al.* 2009; Tariq *et al.* 2010), and there are two possible theories for this. The first is that there may be age-related changes in exposure to parasites, caused by changes in host behaviour (Dalton & Pole 1978; Tinsley 1989) or diet (Thomas 1965; Borgstrom 1970; Martin *et al.* 2005). The second is that there are age-related changes in susceptibility (reducing parasite establishment, survival or fecundity), either resulting from innate changes in immunity (Sreter *et al.* 1995; Robb & Forbes 2006) or immunity acquired through repeated exposure (Crombie & Anderson 1985; Sreter *et al.* 1995; Kabatereine *et al.* 1999; Ladeia-Andrade *et al.* 2009). It is also possible that both factors may be operating together (Anderson 1986). Evidence for acquired immunity can be determined by identifying and comparing 'peak shifts' (Woolhouse 1998) in populations with different transmission rates, that is, the difference in the average age at which intensity peaks between populations (reviewed in Woolhouse 1998; Wilson *et al.* 2002). A population with a high transmission rate will peak at a younger age than a population with a low transmission rate as animals experience sufficient pathogen exposure to develop immunity earlier.

As prevalence, and not intensity, was measured in this study, identifying any peak shifts between different slow worm populations would not have been possible and so, while the age-prevalence curve found for *N. brevicaudatum* in slow worms may indicate acquired immunity, it may also represent changes in exposure with age. Patterns of prevalence do not necessarily match that of intensity (Rózsa *et al.* 2000), and hence age-prevalence curves are potentially more ambiguous than those of intensity as any asymptote or reduction of prevalence in the age-prevalence curve could merely indicate the loss of infected individuals from the population (Sorci

1996). While less powerful, prevalence data is a useful tool for studying epidemiological processes, particularly as it is more readily obtainable using non-invasive techniques such as faecal analysis.

A constraint of non-invasive faecal analysis (whether molecular or traditional) is that it detects only parasites that have been shed and will underestimate prevalence unless all infected animals have shed parasites. However, it can often provide a good estimate of prevalence and is preferable to the invasive alternative of dissection when dealing with vertebrates, particularly protected species of conservation concern.

Here we used a non-invasive molecular approach to model, for the first time, parasite prevalence in a reptile. The application of PCR primers proved to be highly effective at tracking, non-invasively, nematode prevalence in the faeces of reptiles, in this case slow worms. A constraint of non-invasive faecal analysis (whether molecular or traditional) is that it detects only parasites that have been shed and will underestimate prevalence unless all infected animals have shed parasites. However, it can often provide a good estimate of prevalence and is preferable to the invasive alternative of dissection when dealing with vertebrates, particularly protected species of conservation concern. The approach, therefore, has potentially broad application to the study of parasitic infection and host parasite dynamics.

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Author information box

This work was part of the wider PhD project of David Brown, using molecular diagnostic tools to study the diets of adult and juvenile snakes and lizards with a view to their conservation. Bill Symondson runs a research lab dedicated to developing new molecular approaches to the analysis of predator-prey relationships, particularly involving invertebrates, and supervised this project.

Data Accessibility

COI forward and reverse primer sites for *Neoxysomatium brevicaudatum* aligned with other nematode taxa uploaded as online Supporting Information (Table S1). Non-target species tested for cross-reactivity with *Neoxysomatium brevicaudatum* primers COI-J-1764 and COI-N-1938 uploaded as online Supporting Information (Table S2). Details of all 270 slow worms caught uploaded as online Supporting Information (Table S3).

Figure captions

Figure 1. Predicted probability of *Neoxysomatium brevicaudatum* prevalence (with SE bars) in male and female slow worms with months pooled for 2007 and 2008, showing significantly higher prevalence in males than in females in April (*p=0.05*).

Figure 2. Predicted probability of *Neoxysomatium brevicaudatum* prevalence (with SE bars) in male and female slow worms showing significantly lower prevalence in males than in females in August 2007 (*p=0.02*) and July 2008 (*p=0.03*).

Figure 3. Effect of the interaction between slow worm length and weight on the predicted probability of *Neoxysomatium brevicaudatum* prevalence (with SE bars), as found significant in GLM (*p=0.0007*).

Figure 4. Changes in the predicted probability of *Neoxysomatium brevicaudatum* prevalence in slow worms with host length (with SE bars), used as a surrogate for host age. Data shown is calculated for males in May 2008, using average male weights for that month, and is representative of females and of other months.

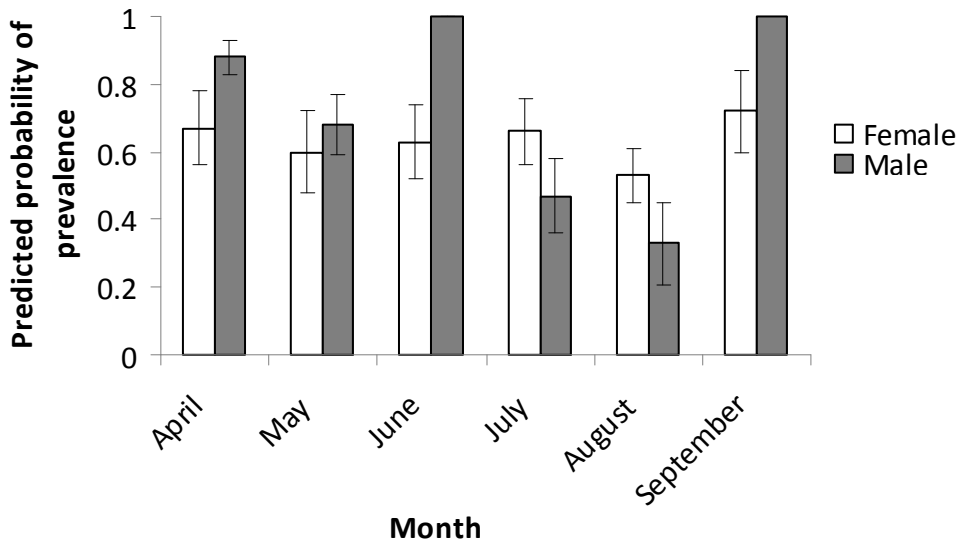
Predicted probability of prevalence
1
0.8
0.6
0.4
0.2
0
April
May
June
July
August
September
Month
Female
Male

Figure 1

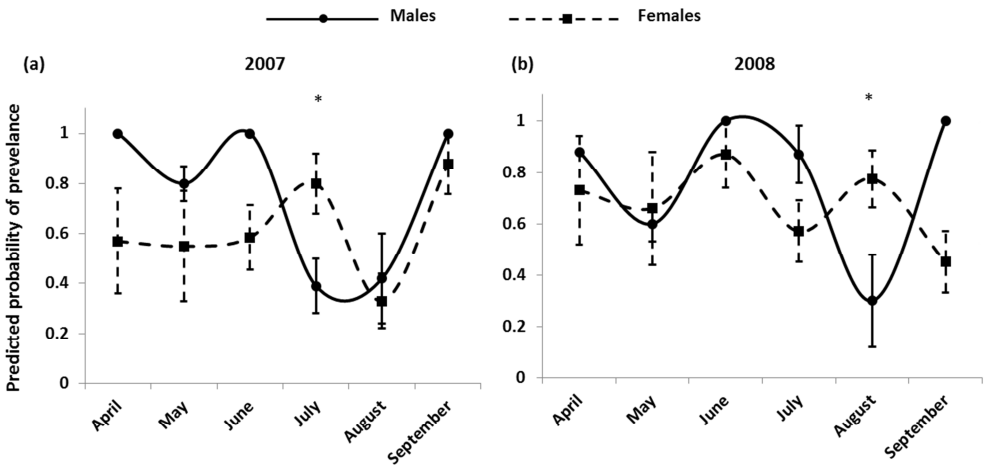
Males
Females
(a)
2007
(b)
2008
Predicted probability of prevelance
0
0.2
0.4
0.6
0.8
1
April
May
June
July
August
September
*
*

Figure 2

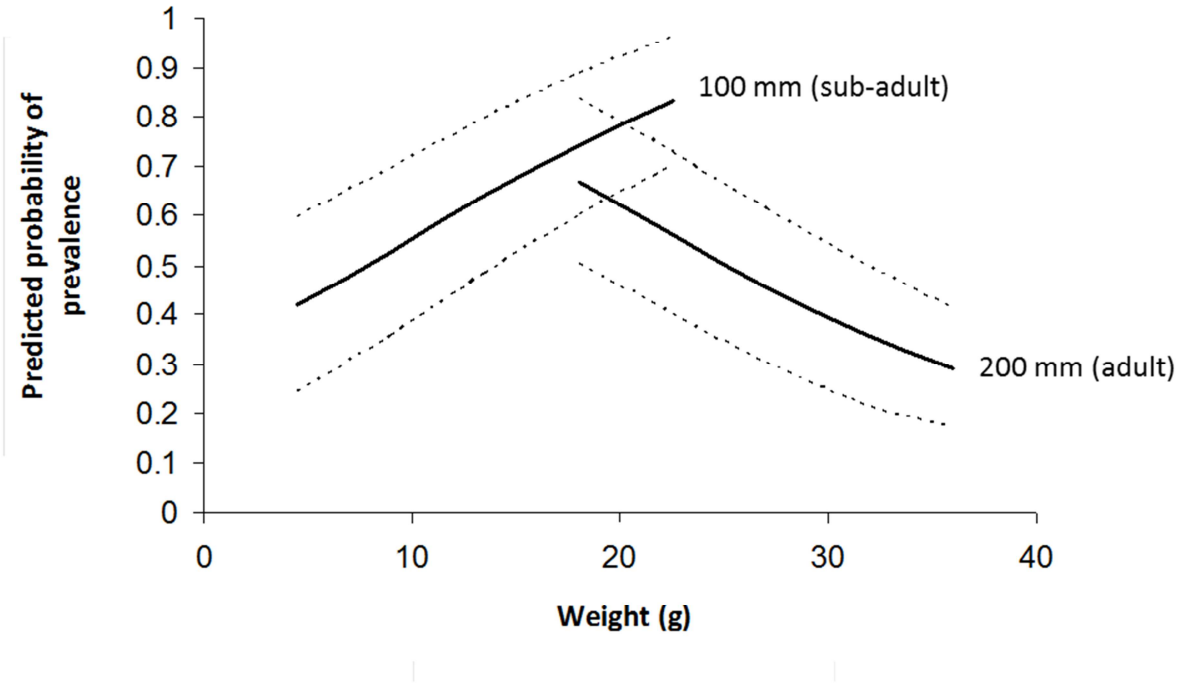
Predicted probability of prevalence
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
Weight (g)
100 mm (sub-adult)
200 mm (adult)

Figure 3

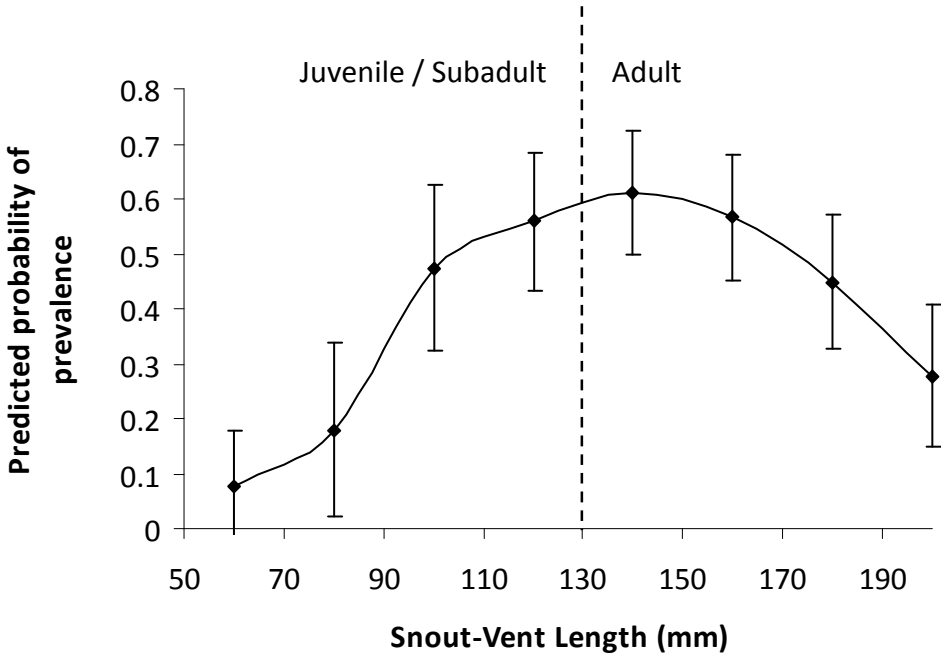
Juvenile / Subadult
Adult
Predicted probability of prevalence
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
50
70
90
110
130
150
170
190
Snout-Vent Length (mm)

Figure 4

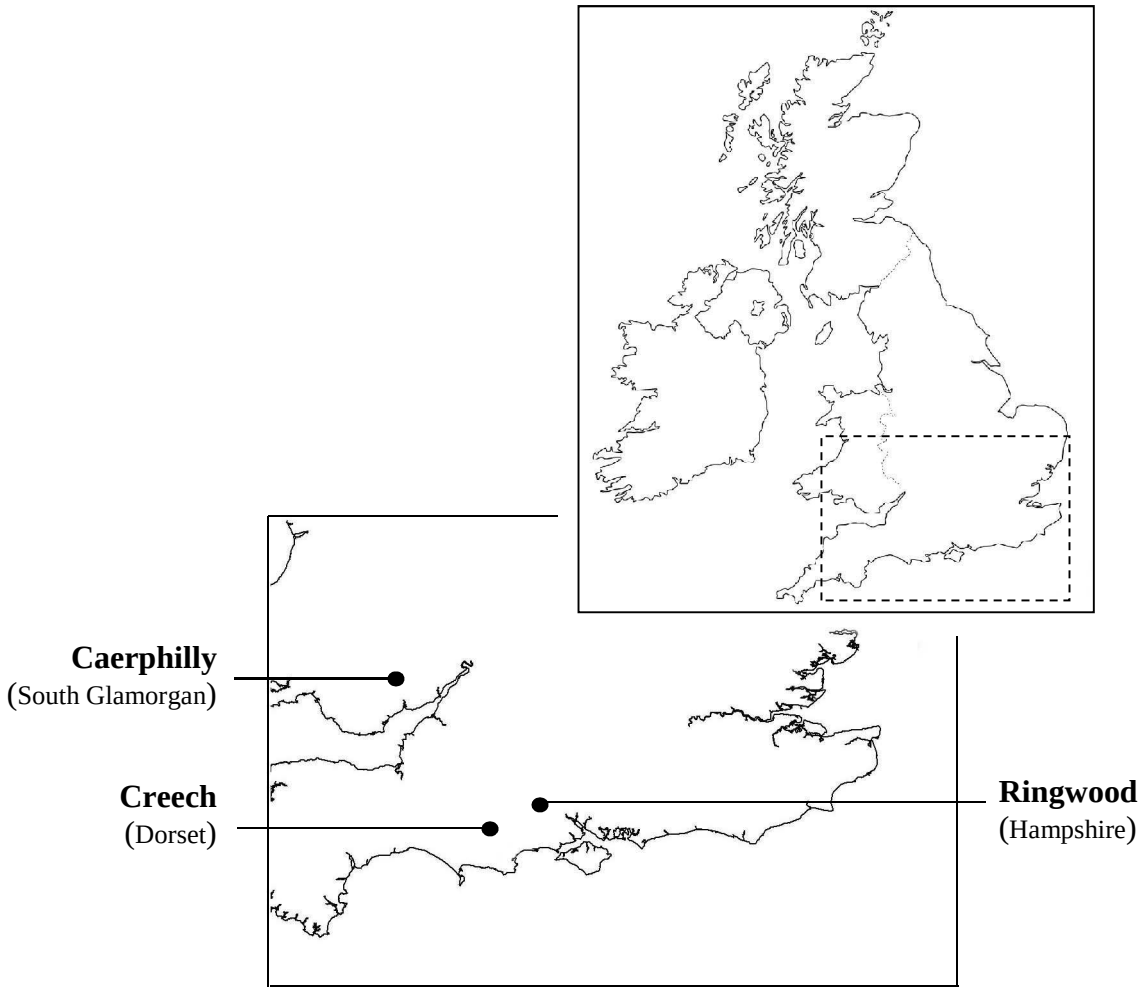

Figure S1. Locations of sampling sites where slow worm faecal samples were collected.

Table S1. Cytochrome Oxidase I forward and reverse primer sites for *Neoxysomatium brevicaudatum* aligned with other nematode taxa.

Nematode species	**Classification**	5' nucleotide position: 1764	5' nucleotide position: 1938
	Ascaridida		
Neoxysomatium bredicaudatum	Cosmocercoidea	5'-TCTTAGATTTTGACTTTTGCCTACAG-3'	3'-CTAATGTAACACGACCACAATCAAGA-5'
Heterakis isolonche (FJ009627)	Heterakoidea	.A....T.....GT.G.....A..TT	.T.......T.......A........
Anisakis simplex (AY994157)	Ascaridoidea	.T.A..T.....GT.A........T.	.T.....C........G........
Toxocara cati (AM411622)	Ascaridoidea	.T.G..T.....GT.G........G.	.A.................C......
Anisakis pegreffii (FJ907317)	Ascaridoidea	C.....C.....GT.G........T.	.AG..........C...........
Rostellascaris sp. (EU741046)	Ascaridoidea	.T.A..T.....GT.A..........	.A.................T......
Ascaris suum (X54253)	Ascaridoidea	.T.A..T.....GT.G........T.	.A..C...........C.....T..N
	Rhabditida		
Necator americanus (AF303157)	Ancylostomatoidea	.T.A..T.....GT.A..A.....T.	.A..............A.T......
Parelaphostrongylus odocoilei (EF173699)	Metastrongyloidea	CT.A.........T.A..A.....TT	................A.T.....T
Steinernema arenarium (AY943979)	Panagrolaimoidea	...A..T..C...T.A..A.....T.	.A...........G...T.......C
Steinernema cubanum (AY943983)	Panagrolaimoidea	.T.A........GT.A..A.....T.	.AG.A........G...T........
Steinernema carpocapsae (AY943981)	Panagrolaimoidea	.T.A..T......T.GC.A..A..T.	.AG.A........T..TT....G..T
Strongylus equinus (U57038)	Strongyloidea	...A..T...G..T.G..A..C..T.	.A..C........T...A..TCA...
Diplogastrellus metamasius (EU419761)	Rhabditoidea	.T.A.........T.A..A..A..T.	.A..............CA.T.....T
Oigolaimella sp. (AB478634)	Rhabditoidea	.T.A.........T.G..A..A..TT	.A..............A.T.....T
Rhabdias okuensis (FM179479)	Rhabditoidea	.A.A..T......T.A..A.......	.A.............T.....C..T
Rhabdias mariauxi (FN395319)	Rhabditoidea	.A.A..T......T.AC.........	.A.............TT....C..T
Mecistocirrus digitatus (AB245051)	Trichostrongyloidea	.T.G..T......T.G..A.....G.	.A..............A..TCT...
Haemonchus contortus (EU346694)	Trichostrongyloidea	.T.A.........T.G..A.....T.	.AG.A........C...A..TCT..T
Nippostrongylus brasiliensis (U57035)	Trichostrongyloidea	.T.A..T......T.A..A.....T.	.A.........A.C...A..TCA..T
Heligmosomum mixtum (DQ408635)	Trichostrongyloidea	.T.G..T.....GT.A..A..A..T.	................A..TCA..T
	Tylenchida		
Bursaphelenchus poligraphi (AY508059)	Aphelenchoididae	.T.A.....C...T.A..A.....TT	.A..C...........C...TCA.A.
Bursaphelenchus seani (AY508061)	Aphelenchoididae	.T.A.............A....TTT	.AG.A...........C...TCA..T

Table S2. Non-target species tested for cross-reactivity with *Neoxysomatium brevicaudatum* primers COI-J-1764 and COI-N-1938.

Species	**Phylum**	**Order**	**Family**
Anguis fragilis	Chordata	Sauria	Anguidae
Coronella austriaca	Chordata	Squamata	Colubridae
Lumbricus rubellus	Annelida	Haplotaxida	Lumbricidae
Aporrectodea caliginosa	Annelida	Haplotaxida	Lumbricidae
Aporrectodea longa	Annelida	Haplotaxida	Lumbricidae
Lumbricus terrestris	Annelida	Haplotaxida	Lumbricidae
Arion intermedius	Mollusca	Pulmonata	Arionidae
Arion owenii	Mollusca	Pulmonata	Arionidae
Arion hortensis	Mollusca	Pulmonata	Arionidae
Arion distinctus	Mollusca	Pulmonata	Arionidae
Limax flavus	Mollusca	Pulmonata	Limacidae
Forficula sp.	Arthropoda	Dermaptera	Forficulidae
Erigone dentipalpis	Arthropoda	Araneae	Linyphiidae
Formicidae sp.	Arthropoda	Hymenoptera	Formicidae
Tipulidae sp.	Arthropoda	Diptera	Tipulidae
Notiophilus biguttaus	Arthropoda	Coleoptera	Carabidae
Adalia bipunctata	Arthropoda	Coleoptera	Coccinellidae
Tachyporus obtusus	Arthropoda	Coleoptera	Staphylinidae
Phasmarhabditis hermaphrodita	Nematoda	Rhabditida	Rhabditidae
Steinernema feltiae	Nematoda	Rhabditida	Steinernematidae
Rhabdias spp.	Nematoda	Rhabditida	Rhabdiasidae
Oswaldocruzia filiformis	Nematoda	Ascaridida	Molineidae

Table S3. Details of slow worms (*N=270*) screened for presence/absence of *Neoxysomatium brevicaudatum*.

Year	Month	Mean Temp (°C)	Rainfall (mm)	Site	Sex	Age	SVL (mm)	WT (g)	Tail (presence/absence)	Neoxysomatium (presence/absence)
2007	April	17	6.1	Noon	Female	Sub	87	10	1	0
2007	April	17	6.1	Noon	Female	Adult	140	21.2	1	0
2007	April	17	6.1	Noon	Female	Sub	115	13	1	1
2007	April	17	6.1	Noon	Female	Sub	125	9.1	0	1
2007	April	17	6.1	Noon	Female	Adult	135	18.5	1	0
2007	April	17	6.1	Noon	Male	Adult				1
2007	April	17	6.1	Noon	Male	Sub	110	11	1	1
2007	April	17	6.1	Noon	Male	Adult	160	23.1	0	1
2007	April	17	6.1	Noon	Male	Adult	130	24.3	1	1
2007	April	17	6.1	Noon	Male	Adult	150			0
2007	August	19.4	77	Caerphilly	Female	Adult	150	18.6	0	0
2007	August	19.4	77	Caerphilly	Female	Adult	170	27	1	0

2007	August	19.4	77	Caerphilly	Female	Adult				1
2007	August	20.6	53.7	Creech	Female	Sub	125	14	1	0
2007	August	20.6	53.7	Creech	Female	Adult	135	19	1	0
2007	August	20.6	53.7	Creech	Female	Adult	135	20	1	0
2007	August	20.6	53.7	Noon	Female	Adult	150	19	1	0
2007	August	20.6	53.7	Noon	Female	Adult	150	23	1	0
2007	August	20.6	53.7	Noon	Female	Adult	170	24	1	0
2007	August	20.6	53.7	Noon	Female	Adult	175	26	1	0
2007	August	20.6	53.7	Noon	Female	Adult				1
2007	August	20.6	53.7	Noon	Female	Adult	130	17	1	0
2007	August	20.6	53.7	Noon	Female	Adult	150	19	0	1
2007	August	20.6	53.7	Noon	Female	Adult	150	22	0	1
2007	August	20.6	53.7	Noon	Female	Adult	150	24	1	0
2007	August	20.6	53.7	Noon	Female	Adult	160	21	1	0
2007	August	20.6	53.7	Creech	Female	Adult	165	21	0	1
2007	August	20.6	53.7	Noon	Female	Adult	165	24.1	0	1

2007	August	20.6	53.7	Creech	Female	Adult	173	22.6	1	1
2007	August	20.6	53.7	Creech	Female	Adult	178			1
2007	August	20.6	53.7	Creech	Female	Sub	115		1	0
2007	August	20.6	53.7	Creech	Female	Adult	140	14	1	0
2007	August	20.6	53.7	Noon	Female	Adult	140	18	1	0
2007	August	20.6	53.7	Creech	Female	Adult	145	17	1	1
2007	August	20.6	53.7	Noon	Female	Adult	150	20	1	1
2007	August	20.6	53.7	Creech	Female	Adult	160	18.4	0	0
2007	August	19.4	77	Caerphilly	Male	Adult	160	22	1	1
2007	August	19.4	77	Caerphilly	Male	Adult	160	21.5	1	0
2007	August	19.4	77	Caerphilly	Male	Adult	160	25.2	1	1
2007	August	19.4	77	Caerphilly	Male	Adult	170		0	1
2007	August	20.6	53.7	Noon	Male	Sub	125	17	1	0
2007	August	20.6	53.7	Noon	Male	Adult	145	19	0	0
2007	August	20.6	53.7	Noon	Male	Sub	110	14	1	1
2007	August	20.6	53.7	Noon	Male	Adult	135	18	1	0

2007	August	20.6	53.7	Creech	Male	Adult	180	24.9	1	0
2007	July	18.5	146.7	Caerphilly	Female	Adult	185	28	0	0
2007	July	19.8	121.7	Noon	Female	Sub	120	12	1	0
2007	July	19.8	121.7	Noon	Female	Adult	155	24	1	1
2007	July	19.8	121.7	Noon	Female	Adult	155	26	1	1
2007	July	19.8	121.7	Noon	Female	Adult	155		1	1
2007	July	19.8	121.7	Noon	Female	Adult	160	21.1	0	1
2007	July	19.8	121.7	Noon	Female	Adult	140	19.2	1	0
2007	July	19.8	121.7	Noon	Female	Adult	145	15	1	1
2007	July	19.8	121.7	Noon	Female	Adult	170	26	1	1
2007	July	19.8	121.7	Creech	Female	Sub	108	12	1	1
2007	July	19.8	121.7	Creech	Female	Sub	120	20	1	1
2007	July	19.8	121.7	Creech	Female	Adult	140	15	0	1
2007	July	19.8	121.7	Creech	Female	Adult	155	19	1	1
2007	July	19.8	121.7	Creech	Female	Adult	155	19	0	1
2007	July	19.8	121.7	Creech	Female	Adult				1

2007	July	19.8	121.7	Creech	Female	Adult				1
2007	July	19.8	121.7	Noon	Male	Sub	120	14	1	0
2007	July	19.8	121.7	Noon	Male	Adult	140	20	1	1
2007	July	19.8	121.7	Noon	Male	Adult	160	15.1	0	0
2007	July	19.8	121.7	Noon	Male	Adult	170	25	0	1
2007	July	19.8	121.7	Noon	Male	Adult	142	17.6	1	0
2007	July	19.8	121.7	Noon	Male	Adult	145	20	0	0
2007	July	19.8	121.7	Noon	Male	Adult	155	18	1	0
2007	July	19.8	121.7	Noon	Male	Adult	160	24	1	0
2007	July	19.8	121.7	Noon	Male	Adult	175	26	1	0
2007	July	19.8	121.7	Creech	Male	Sub	120	17	1	0
2007	July	19.8	121.7	Creech	Male	Sub	124	15	1	1
2007	July	19.8	121.7	Creech	Male	Adult	133	17.8	1	1
2007	June	19.6	123.8	Noon	Female	Adult	160			0
2007	June	19.6	123.8	Noon	Female	Sub	100	9	1	0
2007	June	19.6	123.8	Noon	Female	Sub	110	7	1	0

2007	June	19.6	123.8	Creech	Female	Adult	130	12.5	1	1
2007	June	19.6	123.8	Noon	Female	Adult	140	17	1	0
2007	June	19.6	123.8	Creech	Female	Adult	140	18	0	0
2007	June	19.6	123.8	Noon	Female	Adult	140	19	1	1
2007	June	19.6	123.8	Noon	Female	Adult	160	26.7	1	1
2007	June	19.6	123.8	Noon	Female	Adult	165	25	1	0
2007	June	19.6	123.8	Noon	Female	Adult	170	25	0	1
2007	June	18.5	146.7	Caerphilly	Female	Sub	95	4	1	1
2007	June	18.5	146.7	Caerphilly	Female	Adult	150	24	1	0
2007	June	18.1	165.1	Caerphilly	Female	Adult	140	17	0	1
2007	June	18.1	165.1	Caerphilly	Female	Adult	160	23	0	1
2007	June	18.5	146.7	Caerphilly	Female	Adult	155	20	0	0
2007	June	18.5	146.7	Caerphilly	Female	Adult	175	23.4	0	0
2007	June	19.6	123.8	Creech	Male	Sub	115	11	1	1
2007	June	18.5	146.7	Caerphilly	Male	Sub	110	10.5	1	1
2007	June	19.6	123.8	Noon	Male	Adult	165		1	1

2007	June	19.6	123.8	Noon	Male	Adult	180	25	0	1
2007	May	15.9	138.1	Caerphilly	Female	Sub	110		0	0
2007	May	16.6	119.4	Noon	Female	Sub	120	12.5	1	0
2007	May	16.6	119.4	Noon	Female	Adult	130	15	1	0
2007	May	16.6	119.4	Noon	Female	Adult	135	18	1	1
2007	May	16.6	119.4	Creech	Female	Adult	135	18	1	1
2007	May	15.9	138.1	Caerphilly	Male	Sub	94			1
2007	May	15.9	138.1	Caerphilly	Male	Adult	130	13.5	1	0
2007	May	15.9	138.1	Caerphilly	Male	Sub	105	12.5	1	0
2007	May	16.6	119.4	Creech	Male	Sub	95	7.5	1	1
2007	May	16.6	119.4	Creech	Male	Sub	100	10	1	1
2007	May	16.6	119.4	Creech	Male	Adult	130	12.5	1	1
2007	May	16.6	119.4	Creech	Male	Adult	130	14	1	1
2007	May	16.6	119.4	Creech	Male	Adult	130	16	1	1
2007	May	16.6	119.4	Noon	Male	Adult	135	14.5	0	1
2007	May	16.6	119.4	Noon	Male	Adult	150	16.5	1	1

2007	May	16.6	119.4	Noon	Male	Adult	160	16	0	1
2007	May	16.6	119.4	Noon	Male	Adult	165	21	0	1
2007	May	16.6	119.4	Noon	Male	Adult	180	25.7	0	0
2007	May	16.6	119.4	Noon	Male	Adult	135	14.5	1	1
2007	May	16.6	119.4	Creech	Male	Adult	140	15	0	0
2007	September	17.7	60	Caerphilly	Female	Adult	170	27	1	1
2007	September	18.6	35.1	Creech	Female	Adult	160	17.1	1	1
2007	September	18.6	35.1	Noon	Female	Adult	160	20	0	1
2007	September	18.6	35.1	Noon	Female	Adult	165	16	1	1
2007	September	18.6	35.1	Noon	Female	Adult	170	18	1	1
2007	September	18.6	35.1	Creech	Female	Sub	120	7.4	1	1
2007	September	18.6	35.1	Noon	Female	Adult	140	21	1	1
2007	September	18.6	35.1	Noon	Female	Adult	145	23	1	0
2007	September	18.6	35.1	Creech	Female	Adult	168			1
2007	September	17.7	60	Caerphilly	Male	Sub	110	13	1	1
2007	September	18.6	35.1	Noon	Male	Adult	155	17	0	1

2007	September	18.6	35.1	Noon	Male	Adult				1
2008	April	11.9	72.2	Caerphilly	Female	Adult	170		0	0
2008	April	12.6	57.5	Noon	Female	Sub	110		0	1
2008	April	12.6	57.5	Noon	Female	Sub	110		1	0
2008	April	12.6	57.5	Noon	Female	Sub	125	20	1	1
2008	April	12.6	57.5	Noon	Female	Adult	130	25	1	1
2008	April	12.6	57.5	Noon	Female	Adult	130	26	1	0
2008	April	12.6	57.5	Noon	Female	Adult	140	27	1	1
2008	April	12.6	57.5	Noon	Female	Adult	150	22	1	1
2008	April	12.6	57.5	Noon	Female	Adult	150	29	1	1
2008	April	12.6	57.5	Noon	Female	Adult	160	35	1	1
2008	April	12.6	57.5	Noon	Female	Adult	165	25	1	0
2008	April	12.6	57.5	Noon	Female	Adult	170	30	1	1
2008	April	12.6	57.5	Creech	Female	Sub	125	12	1	0
2008	April	12.6	57.5	Creech	Female	Sub	128	15	1	1
2008	April	12.6	57.5	Creech	Female	Adult	130	20	1	1

2008	April	12.6	57.5	Creech	Female	Adult	145	21	1	1
2008	April	12.6	57.5	Creech	Female	Adult	145		0	0
2008	April	12.6	57.5	Creech	Female	Adult	165	27	1	0
2008	April	12.6	57.5	Creech	Female	Adult	173	18	0	0
2008	April	11.9	72.2	Caerphilly	Male	Sub	127		1	1
2008	April	11.9	72.2	Caerphilly	Male	Adult	148	32	0	1
2008	April	11.9	72.2	Caerphilly	Male	Adult	150		1	1
2008	April	11.9	72.2	Caerphilly	Male	Adult	150		0	1
2008	April	11.9	72.2	Caerphilly	Male	Adult	152		1	1
2008	April	11.9	72.2	Caerphilly	Male	Adult	160		1	1
2008	April	11.9	72.2	Caerphilly	Male	Adult	180	31	0	1
2008	April	12.6	57.5	Noon	Male	Sub	110	25	1	1
2008	April	12.6	57.5	Noon	Male	Sub	120	20	1	0
2008	April	12.6	57.5	Noon	Male	Sub	120	20	1	1
2008	April	12.6	57.5	Noon	Male	Adult	130	25	1	1
2008	April	12.6	57.5	Noon	Male	Adult	130		0	1

2008	April	12.6	57.5	Noon	Male	Adult	140	25	0	1
2008	April	12.6	57.5	Noon	Male	Adult	140	27	1	1
2008	April	12.6	57.5	Noon	Male	Adult	140	30	1	1
2008	April	12.6	57.5	Noon	Male	Adult	150	30	1	1
2008	April	12.6	57.5	Noon	Male	Adult	150	31	1	1
2008	April	12.6	57.5	Noon	Male	Adult	150	32	0	0
2008	April	12.6	57.5	Noon	Male	Adult	170	35	1	1
2008	April	12.6	57.5	Creech	Male	Sub	125	15	1	1
2008	April	12.6	57.5	Noon	Male	Adult	140	20	1	1
2008	April	12.6	57.5	Creech	Male	Adult	145	20	1	1
2008	April	12.6	57.5	Creech	Male	Adult	145	20	0	1
2008	April	12.6	57.5	Creech	Male	Adult	150	20	0	1
2008	April	12.6	57.5	Noon	Male	Adult	150	20	1	1
2008	April	12.6	57.5	Noon	Male	Adult	165	30	1	0
2008	April	12.6	57.5	Noon	Male	Adult	188	30	1	1
2008	August	20.2	92.8	Noon	Female	Adult	140	13.1	0	1

2008	August	20.2	92.8	Noon	Female	Adult	150	20.5	1	1
2008	August	20.2	92.8	Noon	Female	Adult	160	21.5	1	1
2008	August	20.2	92.8	Noon	Female	Adult	160	25.7	1	1
2008	August	20.2	92.8	Noon	Female	Adult	160		1	0
2008	August	20.2	92.8	Creech	Female	Adult	135	13.8	1	1
2008	August	20.2	92.8	Noon	Female	Adult	140	11.2	0	0
2008	August	20.2	92.8	Noon	Female	Adult	140	12	0	0
2008	August	20.2	92.8	Noon	Female	Adult	140		1	1
2008	August	21.2	90.5	Creech	Female	Adult	145	16.2	1	1
2008	August	21.2	90.5	Creech	Female	Adult	150	14.2	1	1
2008	August	20.2	92.8	Noon	Female	Adult	200	28	0	0
2008	August	18.3	165.5	Caerphilly	Female	Sub	120	6.6	1	0
2008	August	18.3	165.5	Caerphilly	Female	Adult	160	17.3	0	1
2008	August	18.3	165.5	Caerphilly	Female	Adult	170	21	0	1
2008	August	18.3	165.5	Caerphilly	Female	Adult	195	35.3	1	0
2008	August	20.2	92.8	Noon	Female	Adult	130	13	1	1

2008	August	20.2	92.8	Noon	Female	Adult	180		1	1
2008	August	20.2	92.8	Noon	Female	Adult	200	23.2	0	0
2008	August	20.2	92.8	Noon	Female	Sub	125	8.2	0	1
2008	August	20.2	92.8	Creech	Female	Adult	150	18	0	1
2008	August	20.2	92.8	Noon	Female	Adult	155	18.5	1	1
2008	August	20.2	92.8	Noon	Male	Sub	120	11.4	1	1
2008	August	20.2	92.8	Noon	Male	Adult	140	21.1	1	0
2008	August	20.2	92.8	Noon	Male	Adult	140	13.7	1	0
2008	August	20.2	92.8	Noon	Male	Adult	145	12.8	1	0
2008	August	20.2	92.8	Noon	Male	Adult	150	13.3	0	0
2008	August	21.2	90.5	Creech	Male	Adult	155	19.6	1	1
2008	August	18.3	165.5	Caerphilly	Male	Sub	120	9.6	0	0
2008	August	20.2	92.8	Creech	Male	Adult	155	18	1	0
2008	August	20.2	92.8	Noon	Male	Adult	175	28	0	0
2008	July	19.3	158.3	Caerphilly	Female	Adult	180	28.6	1	0
2008	July	19.3	158.3	Caerphilly	Female	Adult	210	25.2	0	0

2008	July	19.3	158.3	Caerphilly	Female	Adult	160	23	1	0
2008	July	19.3	158.3	Caerphilly	Female	Adult	190	31	0	0
2008	July	19.3	158.3	Caerphilly	Female	Adult	200	33.6	1	1
2008	July	21.2	90.5	Noon	Female	Adult	170	21.2	1	0
2008	July	21.2	90.5	Creech	Female	Adult	130	10.8	0	0
2008	July	21.2	90.5	Creech	Female	Adult	150	19.5	1	1
2008	July	21.2	90.5	Creech	Female	Adult	160	18	1	1
2008	July	21.2	90.5	Noon	Female	Adult	160	22	1	1
2008	July	21.2	90.5	Noon	Female	Adult	160	24	0	1
2008	July	21.2	90.5	Noon	Female	Adult	180	28	0	0
2008	July	19.3	158.3	Caerphilly	Male	Sub	120	12.3	1	1
2008	July	21.2	90.5	Noon	Male	Adult	150		1	1
2008	July	21.2	90.5	Noon	Male	Adult	145	13.5	0	1
2008	July	21.2	90.5	Noon	Male	Adult	210	35	1	0
2008	June	21.2	90.5	Noon	Female	Adult	160		1	1
2008	June	19.1	44.4	Creech	Female	Sub	122	10.5	1	0

2008	June	19.1	44.4	Noon	Female	Adult	160	17.5	0	0
2008	June	19.1	44.4	Noon	Female	Adult	160	23.4	1	1
2008	June	19.1	44.4	Creech	Female	Adult	190	20	1	1
2008	June	19.1	44.4	Noon	Female	Sub	120	13.2	0	1
2008	June	19.1	44.4	Noon	Female	Adult	160	18	0	1
2008	June	19.1	44.4	Noon	Female	Adult	165		0	0
2008	June	19.1	44.4	Noon	Female	Adult	170	25	1	1
2008	June	19.1	44.4	Noon	Female	Adult	195	24	0	1
2008	June	19.1	44.4	Noon	Male	Sub	100	23.3	1	1
2008	June	19.1	44.4	Creech	Male	Adult	140		1	0
2008	May	18.3	79.8	Noon	Female	Sub	85		0	0
2008	May	18.3	79.8	Noon	Female	Adult	135	14.2	1	1
2008	May	18.3	79.8	Noon	Female	Adult	150	29	0	1
2008	May	18.3	79.8	Noon	Female	Adult	155	21.2	1	0
2008	May	18.3	79.8	Noon	Female	Adult	160	21.9	1	1
2008	May	18.3	79.8	Noon	Female	Adult	170	18.5	0	1

2008	May	18.3	79.8	Noon	Female	Adult	170	19.5	0	1
2008	May	18.3	79.8	Noon	Female	Adult	170	25	0	0
2008	May	18.3	79.8	Noon	Female	Adult	175	17	1	0
2008	May	18.3	79.8	Noon	Female	Adult	185	33.5	1	0
2008	May	18.3	79.8	Noon	Female	Adult	150	22	1	1
2008	May	17.7	82	Noon	Male	Adult	140	7.4	1	0
2008	May	12.6	57.5	Noon	Male	Sub	110	6	1	0
2008	May	12.6	57.5	Noon	Male	Sub	110	22	1	0
2008	May	12.6	57.5	Noon	Male	Sub	113		1	1
2008	May	12.6	57.5	Noon	Male	Adult	136		I	1
2008	May	12.6	57.5	Noon	Male	Adult	140	14	1	1
2008	May	17.8	61.4	Creech	Male	Sub	112	10	1	0
2008	May	17.8	61.4	Caerphilly	Male	Adult	165	21.8	1	1
2008	May	18.3	79.8	Noon	Male	Sub	110	6	0	0
2008	May	18.3	79.8	Noon	Male	Adult	160	17.3	0	1
2008	May	18.3	79.8	Noon	Male	Adult	170	21	0	0

2008	May	18.3	79.8	Noon	Male	Adult	190	30.1	0	0
2008	May	18.3	79.8	Noon	Male	Adult	210	17.8	0	1
2008	May	18.3	79.8	Noon	Male	Adult	135	12.5	1	1
2008	May	18.3	79.8	Creech	Male	Adult	145	9	0	1
2008	May	18.3	79.8	Creech	Male	Adult	150	9	0	1
2008	May	18.3	79.8	Noon	Male	Adult	160	21	1	1
2008	May	18.3	79.8	Noon	Male	Adult	165	20.4	0	0
2008	May	18.3	79.8	Noon	Male	Adult	175	18.8	0	1
2008	September	17.7	82	Noon	Female	Sub	120	4	1	0
2008	September	17.7	82	Creech	Female	Sub	110		0	1
2008	September	17.7	82	Creech	Female	Adult	130	8.6	1	0
2008	September	17.7	82	Noon	Female	Adult	190	11.4	0	1
2008	September	17.7	82	Creech	Female	Adult	150	7.7	0	0
2008	September	16.9	125.4	Caerphilly	Female	Adult	190	21	1	1
2008	September	17.7	82	Noon	Male	Adult	140	8	1	1
2008	September	17.7	82	Creech	Male	Adult	160	8.3	0	1

	2008	September	16.9	125.4	Caerphilly	Male	Adult	140	8.7	1		1
	2008	September	16.9	125.4	Caerphilly	Male	Adult	145	7.7	0		1

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